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Subject: Addendum to the Effects of 2,4-D in a Two-Generation Study on Reproduction in Rats: Correction on the Histopathology of the Kidneys of Males

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An Addendum to the 2-generation rat reproduction feeding study on 2,4-D has been reviewed(accession # 265489). Only the LEL for the F0 generation changed. The LEL now includes kidney pathology as well as body weight gain reduction. The recalulated and nominal NOEL's and the LEL's with their respective effects are as follows.

F0 parental toxicity.
NOEL - 5(5) mg/kg/day.*
LEL - 20(20) mg/kg/day, male kidney pathology.

F1 parental toxicity.
NOEL - 4(5) mg/kg/day.
LEL - 14(20) mg/kg/day, male kidney pathology, and reduced female body weight.

Developmental toxicity, dose level to dams.
NOEL - 7(5) mg/kg/day.
LEL - 26(20) mg/kg/day, reduced weight in F1b pups.

Nominal dose levels administered 0, 5, 20, or 80 mg/kg/day.

* Calculated lowest dose level within the range consumed by the animals at the nominal dose level administered (nominal dose level administered). The values have been rounded off to the nearest whole number.

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DATA EVALUATION REPORT

STUDY TYPE: Addendum to the study of 2,4-D on Two-Generations of Reproduction in Rats: Correction to histopathology of the kidneys.

TEST SUBSTANCE: 2,4-Dichlorophenoxyacetic Acid (2,4-D)

SYNONYMS: 2,4-D TOX. CHEM. NO. 315

ACCESSION NO.: 265489.

SPONSOR: Industry Task Force on 2,4-D Research Data (ITF)

TESTING FACILITY: Wil Research Laboratories, Inc. (WIL)
Ashland, OH 44805-9281

TITLE OF REPORT: A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxy-acetic Acid: Addendum to the final report.

AUTHORS: Dean E Rodwell, and W. Ray Brown.

STUDY NO.: WIL-81137, same study no. as accession number 265489.

TESTING PERIOD: November 16, 1982 to May 15, 1984.

REPORT ISSUED: September 30, 1986.

PURITY OF TEST SUBSTANCE: See original review of accession no. 259442-6.

CORE GRADE: Not applicable.

CONCLUSIONS ON THE EFFECT AND NO EFFECT LEVELS:

The effect levels for the F0 and F1 males were altered but the no effect levels described in the review of the original study are not altered by the results submitted in this addendum. The LEL and NOEL are restated on the following page. They include kidney histopathological findings reported in this addendum.

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LEL and NOEL is expressed in mg/kg/day(Nominal dose level in mg/kg/day).

F0 parental toxicity
LEL- 19.9(20), degeneration of male kidney tubules.
NOEL- 5(5)
F1 parental toxicity
LEL- 14(20), kidney histopathology in males, and reduced body weight in females.
NOEL- 3.8(5)
Developmental toxicity
LEL- 26(20), F1b pup weight reduction.
NOEL- 7.2(5)

Target or nominal dose levels administered in the study are 0, 5, 20, or 80 mg/kg/day.

CONCLUSIONS:

The reexamination of the kidneys from the 2-generation study on reproduction indicated tubule degeneration in males of the F0 and F1 generations which apparently had not yet developed in 28 day old pups. Cortical tubule degeneration(observed mostly in the proximal convoluted tubules) was confined to the F0 males nominally dosed at 80 mg/kg/day, probably because no F1 animals were dosed at this level passed weaning. Most of these pups died prior to weaning; thus, the study was not continued past weaning. No test substance related kidney histopathology was observed in the remaining pups at any dose level. Both the F0 and the F1 male generations nominally dosed at 20 mg/kg/day demonstrated minimal degeneration of tubules in the outer medullary region of the kidney, but not in the cortical region. No test substance related effects occurred at the nominal dose level of 5 mg/kg/day.

These kidney findings on reexamination cast doubt on the quality of the histological examination conducted in the study on reproduction previously reviewed.

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A. MATERIALS AND METHODS:

Kidney sections prepared on male rats from the F0 and F1 generations and the F1b pups dosed in the two-generation study of the effects of 2,4-D on reproduction in rats were reexamined.

Target or nominal dose levels(mg/kg/day)	Number of rats reexamined (Tissue sections from these animals) F0	F1	F1b
0 (Control)	30	29	10
5 (LDT)	29	30	10
20 (MDT)	30	29	10
80 (HDT)	30	0	14

F0 males were dosed approximately 40 weeks prior to sacrifice. F1 males were dosed approximately 47 weeks, including 3 weeks in utero and 4 weeks of lactation form the milk and from the mothers food supply especially during the last half of the lactaction period. F1b pups(from which the F1 generation was formed) were dosed for 7 weeks as indicated above, 3 weeks in utero and 4 weeks of lactation. The HDT F1 generation males were not reexamined because of poor survival at this dose level.

B. RESULTS:

The results of the histological reevaluation of the male kidneys are presented in Table 1. Tubules of outer medullary region were characterized in the report as demonstrating probable degenerative or atrophic changes of the epithelial cells in the mid and high dose groups. The involved segments were small and the appearance of increased nuclear density was the result of condensation of the effected portions of the tubule.

In addition to the medullary involvement the cortical tubules (mostly the proximal convoluted tubules) of the high dose F0 generation were large and demonstrated a dense, eosinophilic cytoplasm. The lumens of some of these tubules were indistinct when compared to controls. No F1 adult animals were studied at the highest dose level because of death due to excessive toxicity.

In the mid dose, the histology of the kidneys tubules from F0 and F1 males was less clear, but 7/30 F0 animals were reported to demonstrated the increased nuclear density and 4/29 F1 male rats demonstrated similar histopathology(Table 1).

Other sporatic effects occurred in the kidneys with no apparent dose related response.

C. DISCUSSION:

The kidneys of the F0 male rats from the high dose group in the study of reproduction demonsrated degenerative changes in the tubules of the cortical region and the outer medullary region. In the mid dose groups of the F0 generation and the F1 generation (the highest dose level studied in the F1 generation), less distinct changes occurred and they occurred only in the medullary tubules. No dose related kidney effects were seen in the F1b pups at any dose level.

Thus, no effect level for the study on reproduction did not change, however the effect level in adult rats must now include kidney histopathology and reduced weight gain. Prior to the addendum, the lowest effect level was characterized by only a reduced weight gain in adults and pups.

NOTE:

Since this reexamination of the kidney histology was conducted only after the sponsor identified these effects in a rat subchronic study, there is doubt about the quality of the original histological examination conducted in the reproduction study. The reexamination was conducted by Ray Brown of Research Pathology Associates but the original histological examination was conducted by the testing facility, Wil Research Laboratories. Other organs examined histologically by Wil Research but not by Ray Brown are the epidydimis testis, uterus, and ovary. The study on reproduction gave no indication that the kidneys nor any other organ required histological examination.

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Table 1.

Incidence of findings reexamination of kidneys from males
from a 2-generation study of the effects of 2,4-D on reproduction.

Adults or pups	FO adults				F1 adults			F1b pups			
Nominal dose group:	1	2	3	4	1	2	3	1	2	3	4
Number of rats/group:	30	29	30	30	29	30	29	10	10	10	14
Number examined:	30	29	30	30	29	30	29	10	10	10	14
Number normal:	18	21	16	1	12	16	12	9	9	9	11
Description:											
Increased cytoplasmic eosinophilia in cortical tubules.	0	0	0	28	0	0	4	0	0	0	0
Increased focal nuclear density in medullary tubules.											
minimal	0	0	7	17	0	0	4	0	0	0	0
slight	0	0	0	4	0	0	0	0	0	0	0
moderate	0	0	0	1	0	0	0	0	0	0	0
Total incidence	0	0	7	22	0	0	4	0	0	0	0
Multifocal tubular degeneration/basophilia											
minimal	10	8	8	3	13	8	10	1	0	1	3
slight	0	0	0	0	0	2	0	0	0	0	0
Total incidence	10	8	8	3	13	10	10	1	0	1	3
Microcalculi	1	1	1	1	2	4	1	0	1	0	0
Pelvic dilation/hydronephrosis, unilateral.	1	0	1	0	2	1	1	0	0	0	0
Focal/multifocal mononuclear cellular infiltation.	1	1	0	0	3	1	0	0	0	0	0
Focal tubular dilation.	0	0	0	0	3	2	6	0	0	0	0
Focal/multifocal chronic nephritis.	0	0	0	0	1	0	1	0	0	0	0
Pelvic mineralization	0	0	0	0	0	1	0	0	0	0	0
Focal papilllary edema	0	0	0	0	0	0	1	0	0	0	0

Nominal dose groups: 1 = 0 mg/kg/day, 2 = 5 mg/kg/day, 3 = 20 mg/kg/day, 4 = 80 mg/kg/day.

END